

Claims

I claim:

1. A method for sequencing a polynucleotide, comprising the steps of:
  - (i) reacting a target polynucleotide with a polymerase enzyme immobilized on a solid support, and complementary nucleotides, under conditions sufficient for the polymerase reaction; and
  - (ii) detecting an effect consequent on the incorporation of a specific nucleotide complementary to the target polynucleotide by measuring a change in or absorption of applied radiation that occurs during the incorporation.
2. The method according to claim 1, wherein steps (i) and (ii) are conducted with each of the complementary nucleotides in turn, until incorporation is detected, and then repeated.
3. The method according to claim 1, wherein step (i) is conducted with all the complementary nucleotides present.
4. The method according to claim 1, wherein the complementary nucleotides comprise a 3' blocking group which is removed after the polymerase reaction.
5. The method according to claim 4, wherein the blocking group can be selectively removed by pulsed monochromatic light.
6. The method according to claim 4, wherein nucleotides comprise a further blocking group at the terminal phosphate group of the triphosphate chain, and the further blocking group is removed prior to the removal of the 3' blocking group.

7. The method according to claim 6, wherein the further blocking group can be selectively removed by pulsed monochromatic light under conditions different from those required to the 3' blocking group.
8. The method according to claim 7, wherein the further blocking group is removed by pulsing the monochromatic light for a duration different from that required to remove the 3' blocking group.
9. The method according to claim 1, wherein step (i) further comprises introducing a competitive inhibitor of the polymerase enzyme.
10. The method according to claim 1, wherein the target polynucleotide of step (i) is bound to the polymerase enzyme by a  $\beta_2$  dimer complex.
11. The method according to claim 1, wherein the polymerase is an *E. coli* DNA polymerase III or T7 polymerase.
12. The method according to claim 1, wherein the polymerase is a Taq polymerase.
13. The method according to claim 1, wherein the polymerase is a reverse transcriptase.
14. The method according to claim 1, wherein step (ii) comprises detection of a change in resonance signal over time.
15. The method according to claim 1, wherein the radiation is electromagnetic.
16. The method according to claim 15, wherein the electromagnetic radiation is in the infra-red spectrum.

17. The method according to claim 1, wherein the incorporation of a nucleotide is detected using NMR.

18. The method according to claim 1, wherein the polynucleotide is DNA.